Northeast Gulf Science

Volume 7
Number 1 *Number 1*

Article 11

7-1984

Notes on Fecundity of an Arrhenoid Population of Mosquitofish, *Gambusia affinis holbrooki*

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DOI: 10.18785/negs.0701.11

NOTES ON FECUNDITY OF AN ARRHENOID POPULATION OF MOSQUITOFISH, *Gambusia affinis holbrooki*

Howell, *et al.* (1980), provided the first description of an environmentally-induced population of masculinized *Gambusia affinis holbrooki* females exposed to effluent from a pulp and papermill. They described grossly and histologically the gonopodia of the masculinized *G. a. holbrooki* and presented three hypotheses of the possible causes of morphogenesis of the abberant gonopodia. The present study refines, supports and extends the work done by Howell, *et al.* (1980).

This study compares the fecundity of a normal population of *G. a. holbrooki* females with that of an environmentally-induced masculinized population of *G. a. holbrooki* females exposed to effluent from pulp and papermill waste. The normal *G. a. holbrooki* females were collected above the point source of the papermill effluent. The arrehenoid fishes were present only in the main stream, downstream of the point source of the papermill effluent.

This study supports and extends Howell's, *et al.* (1980) hypothesis "that some chemical or combination of chemicals in the papermill effluent is exerting a strong androgenic effect on the Eleven Mile Creek population of *G. a. holbrooki*." Laboratory experiments using androgenic hormones have been shown to cause masculinization in female poeciliids (Turner, 1941, 1942b.; Takahash, 1975; Baldwin, 1942; Eversole, 1941; Vivien, 1950; Hoar, 1969). Other agents and factors also have been shown to induce gonopodial development and to affect fecundity (Blaylock, 1969; Holland, 1973).

This study argues that β-sitosterol, (stigmast-6-en-3β-o1), a sterol which is common in pines, can be transformed by aerobic and/or anaerobic sedimentary microorganisms to form an androgenic hormone such as the two androstane derivatives, 5α-androstan3, 17-dione and androst-4-en-3, 17-dione reported by Taylor, *et al.* (1981).

MATERIALS AND METHODS

G. a. holbrooki were collected by seining during June, July, and August of 1979, June 1980, and March and October of 1982, from areas below and above the papermill effluent of St. Regis Papermill at Eleven Mile Creek at Cantonment, Escambia County, Florida (Howell, *et al.* 1980).

All collections were made with a 1.2m x 1.2m nylon, 3mm mesh seine. Collections were made at random in the main stream downstream from the papermill effluent and upstream from the papermill (Howell, et at. 1980). All fish were killed by fixation in 10% formalin and later preserved in 70% ethanol.

Measurements were made to the nearest millimeter (0.5mm) from the tip of the snout to the caudal peduncle (standard length). All females from all collections were examined for young of the "eyed embryo stage" and the embryos were removed by dissection. Mature eggs were also removed. Actual counts were made on the number of young (eyed embryos) and numbers of mature eggs in the individual broods (Kuntz, 1913). Eggs and embryos were counted and measured on a Nikon compound steromicroscope with an ocular micrometer.

Fecundity was estimated by a linear regression analysis X (standard length) and Y (number of eyed embryos and mature eggs), (Ricker, 1971; Kesteven 1960; Nikolsky, 1963; Sokal, *et al.* 1981).

RESULTS AND DISCUSSION

Collections made in the mainstream below the papermill effluent revealed a population of masculinized *G. a. holbrooki* in which phenotypic females display male secondary sex characters (arrhenoidy). Collections made above the papermill effluent in the mainstream revealed "normal" *G. a. holbrooki* (Howell, *et al.* 1980).

One hundred *G. a. holbrooki* females were randomly selected and examined. Fifty *G. a. holbrooki* females were collected below the papermill effluent and fifty females were collected above the effluent. Of the 50 *G. a. holbrooki* females collected below the effluent 39 displayed male secondary sex character development in the form of gonopodial modification of the anal fin and were gravid, while 11 *G. a. holbrooki* females displayed male secondary sex character development in the form of gonopodial modification and were non-gravid; of the 50 *G. a. holbrooki* females collected above the effluent, 41 were "normal" gravid phenotypic females, while 9 were non-gravid females.

The number of mature eggs in the 39 arrhenoid fishes (17-39mmSL) ranged from 2 to 47. In the 41 normal *G. a. holbrooki* (23-44mmSL) females, the number of mature eggs ranged from 1 to 83. The number of eyed embryos in the 39 arrhenoid fishes (17-39mmSL) ranged from 6 to 59 and in the 41 normal female *G. a. holbrooki* (24-44mmSL) the range was 5 to 104 (Fig. 1).

Variation in fecundity was related primarily to length. The relationship is best described as a linear function of the type:

$$Y = b_0 + b_1x$$

where Y = fecundity and x = the independent variable (length). The data for the arrhenoid fishes is best described by the equation ($R^2 - 0.1815$)

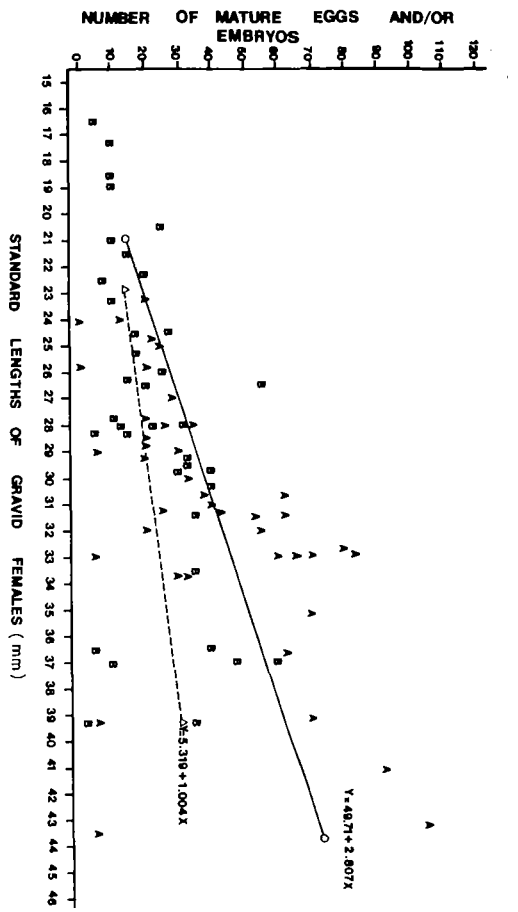

Figure 1. Scatter-plot of the distribution of mature eggs and embryos in relation to maternal length (mm). (A) represents mosquitofish collected above the effluent, (B) represents mosquitofish collected below the effluent. 0 = regression line for the data of normal mosquitofish. Δ = regression line for the data of the arrhenoid mosquitofish.

$$Y = 5.319 + 1.004x$$

The data for the normal *G. a. holbrooki* is best described by the equation ($R^2 - 0.2781$)

$$Y = 49.71 + 2.807x$$

Estimated fecundities in references to length in the arrhenoid fishes were not found to be similar to those found in other studies (Krumholz, 1948; Turner, 1937; Barney and Anson, 1921), although the fecundity of the normal *G. a. holbrooki* were similar.

In Eleven Mile Creek most of the *G. a. holbrooki* collected below the papermill effluent were significantly shorter

DOI: 10.18785/negs.0701.11

than the *G. a. holbrooki* collected above the effluent ($P \leq 0.05$). The larger and more fertile *G. a. holbrooki* females were collected from the sites above the papermill effluent.

When females of similar lengths in the same population were compared, there was some variability in the relationships between the size of the individual female *G. a. holbrooki* and the number of young, and also there were some discrepancies in the regular increase of the average brood size of females of increasing lengths. These irregularities, however, were traceable to the small number of gravid females of certain sizes in the collections. A reason for the diversity in the relationship between the size of the brood and length of the individual female was not readily apparent.

Environmental conditions such as the effluent may account for the increased or decreased rates found in these fish. Recently female fishes (*Heterandria formosa, Poecilia latipinna,* and *Gambusia affinis sp.*) collected from the Fenholloway River, Taylor Country, Florida, downstream of the point source of papermill effluent, displayed the arrhenoid condition previously described by Howell, *et al.* (1980) (Bortone and Drysdale, 1981). Testosterone (methyl and ethyl)-induced gonopodial development has been demonstrated in young female mosquitofish *Gambusia affinis sp.*, the "guppy", *Poecilia reticulatus* and the "swordtail", *Xiphophorus hellerii* (Turner, 1941, 1942a, 1942b.; Essenberg, 1921; Vivien, 1952; Regnier, 1938). Taylor, *et al.* (1981) reported that anaerobic sedimentary environment microorganisms degrade cholesterols (cholest-5-en-3β-o1). Two androstane derivatives, 5α-androstan-3, 17-dione and androst-4-en-3, 17-dione were identified as transformation products. Nitrates were found to be an absolute for the transformation of cholesterol to steriods. Rapid rates of transformation of cholesterol occured only when detectable levels of nitrates were present (Taylor, *et al.* 1981).

The ability to degrade cholesterol, specifically sterols, by aerobic microorganisms of soil and the gastrointestinal tract have been reported (Arima, *et al.* 1969; Marsheck, *et al.* 1972; Nagasawa, *et al.* 1969).

Androstane derivatives which show a low degree of androgenicity such as 5α-androstan-3, 17-dione have been shown to cause partial masculinization of the external genitalia (Krüskemper, 1969).

In addition to the ecological data (BOD, turbidity, pH, DO, COD, etc.), Environmental Protection Agency data on pulp and papermill effulents show that coliforms and other bacteria are present. In these effulents ammonia occurs which can be transformed by nitrifying bacteria to nitrates or by chemical transformations (EPA 440/1-76/047b, 1976). Some organic compounds such as β-sitosterol, a sterol in pines and certain non-degradable organic compounds are present but unmonitored (EPA 440/1-76/047b, 1976).

Therefore, it would be reasonable in hypothesizing that microorganisms (aerobic and/or anaerobic sedimentary bacteria) are transforming β-sitosterol, a sterol common in the pine, *Pinus sp.* (Browning, 1975), into an androgenic hormone.

ACKNOWLEDGEMENTS

The authors would like to thank Drs. M. Howell, J.S. Ramsey, T. Timmons, S. Boney, for their helpful comments and suggestions, Drs. S. Malvestuto and R. Uwate for their helpful comments on the statistical analysis, Dr. D. Shapiro for his detailed reviews, and Mrs. C. Thompson and Mrs. V. Jones for typing. Special

thanks goes to Dr. R. Backus and R. Gagosian. This work was partially completed during a traineeship in Biological Oceanography at Woods Hole Oceanographic Insitution.

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